CLXXIII.—*The Constituents of* Clematis vitalba.

By Frank Tutin and Hubert William Bentley Clewer.

Clematis vitalba, Linné, is a climbing plant which is well known in this country under the name of "Traveller's Joy" or "Old Man's Beard." It is stated in books of reference to contain a volatile, acrid substance, tannic acid, mucilage, and earthy salts, together with an alkaloid, "clematine." On referring, however, to an early publication on the subject (Gaube, *J. Pharm. Chim.*, 1869, **10**, 122), it was found that the author did not claim to have isolated an alkaloid, but only "a product which was alkaline," and no mention is made of its being an organic substance.

Attention has been drawn to the subject of *Clematis* by Dr. F. Baum, who considered it to merit a chemical examination. In view of the very scanty information at present available regarding the constituents of the plant in question, it has been investigated by the present authors.

No alkaloid, and only a trace of volatile substance could be detected, and the statements regarding the acrid, irritant properties of the plant cannot be confirmed. The most interesting substances which have now been isolated are caulosapogenin, $C_{42}H_{66}O_6$, and a new *glucoside* of the latter, which has the properties of a saponin. A summary of the results of the present investigation will be found at the end of this paper.

Experimental.

The material employed for this investigation consisted of the flowering branches of *Clematis vitalba*, Linné, which had been

specially collected by one of the authors near Ware, in Devonshire, during the summer of 1912.

In order to ascertain whether any fugitive, volatile substance were present, as has previously been alleged, 450 grams of the fresh material were cut in small pieces and distilled in a current of steam, but practically no volatile material was removed.

Ten grams of the dried and ground material were digested with Prollius's fluid, and the resulting extract tested for the presence of an alkaloid, but with a negative result.

Twenty grams of the dried and ground material were extracted successively in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 35—50°)	extracted	0·20	gram	= 1·00	per cent.
Ether	,,	0·21	,,	= 1·05	,,
Chloroform	,,	0·11	,,	= 0·55	,,
Ethyl acetate	,,	0·31	,,	= 1·55	,,
Alcohol	,,	1·62	,,	= 8·10	,,
	Total	2·45	grams	= 12·25	per cent.

For the purpose of a complete examination, 55 kilograms of the fresh material were dried, when an amount of moisture was lost equivalent to 64·9 per cent. of the original weight. A quantity (18·35 kilograms) of the dried and ground material was then completely extracted with hot alcohol, when, after the removal of the greater part of the solvent, 4·36 kilograms of a nearly black extract were obtained.

The entire amount of the above-mentioned extract was mixed with water, and steam passed through the mixture for several hours, but nothing appreciable was removed by this treatment. There then remained in the distillation flask a dark brown, aqueous liquid (*A*) and a quantity of dark green resin (*B*). The latter was separated, and repeatedly washed with boiling water, the concentrated washings being added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

Isolation of 3:4-*Dihydroxycinnamic Acid.*

The aqueous liquid (*A*) was extracted six times with ether, the combined ethereal extracts washed, and then shaken successively with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide, the alkaline extracts being immediately acidified. The ammonium carbonate extract yielded brown, resinous material, together with some crystals. The total liquid was, therefore, again extracted with ether, and the ethereal liquid fractionally extracted with many small successive portions of aqueous ammonium

carbonate, when the crystals were obtained almost free from resin. The product so obtained was recrystallised from a mixture of alcohol and ethyl acetate, when it formed small, pale brown prisms, which melted and decomposed at 218°, and proved to be 3 : 4-dihydroxycinnamic acid. (Found, C = 60·0; H = 4·6. Calc., C = 60·0; H = 4·4 per cent.) The amount obtained was 3 grams. 3 : 4-Dihydroxycinnamic acid has frequently been obtained from plant products after treatment with alkali hydroxides, but its occurrence in the free state appears to be rare.

The products obtained from the above-mentioned sodium carbonate and potassium hydroxide extracts consisted essentially of amorphous material, but the former also contained a very small amount of a crystalline substance.

Isolation of Caulosapogenin, $C_{42}H_{66}O_6$.

The original aqueous liquid (*A*) which had been extracted with ether was shaken with ten successive portions of amyl alcohol. The combined amyl alcohol extracts were washed and concentrated under diminished pressure, when, on cooling, a brown-coloured solid separated, and was collected. Both the solid (*a*) and the filtrate (*b*) were mixed with water and evaporated under diminished pressure for the removal of the amyl alcohol, when clear, brown-coloured solutions were obtained. The aqueous solution of the solid (*a*), however, on keeping, slowly deposited a quantity of a brown, tarry product, from which the supernatant liquid was decanted. A portion of the latter was then treated with such an amount of sulphuric acid as to represent 4 per cent. of the total mixture, whereupon the liquid became turbid. Sufficient alcohol was then added to produce a clear solution, and the mixture boiled for two hours, after which it was cooled, and extracted with ether. The ethereal extracts were washed, and extracted successively with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide. The material removed by the first-mentioned alkali was resinous in character, but on acidifying the sodium carbonate and potassium hydroxide extracts and extracting them with ether, a product was obtained which yielded colourless, rhombohedral crystals, on allowing its solution in boiling alcohol to evaporate. This substance melted at 323°, and proved to be identical with caulosapogenin, $C_{42}H_{66}O_6$, a substance which was recently obtained by Power and Salway from *Caulophyllum thalictroides* (T., 1913, **103**, 198). The identity of the two preparations in question was confirmed by direct comparison of the

original products and of their derivatives. The caulosapogenin from *Clematis* yielded the following results:

0·1014 gave 0·2817 CO_2 and 0·0933 H_2O. C = 75·8; H = 10·2.
0·4414, in 23·3 acetic acid, gave $\Delta t + 0·072°$. M.W. = 668.
$C_{42}H_{66}O_6$ requires C = 75·7; H = 9·9 per cent. M.W. = 666.

Diacetylmonosodiocaulosapogenin was also prepared. (Found, Na = 3·7. $C_{46}H_{69}O_8Na$ requires Na = 3·0 per cent.)

It having been ascertained that caulosapogenin is unchanged by boiling for five hours with concentrated alcoholic potassium hydroxide, the action of this alkali on the above-described diacetyl-sodio-derivative (prepared by Power and Salway) was investigated, when it was found that caulosapogenin was recovered unchanged. It therefore appears probable that no more profound change than acetylation occurs during the treatment with acetic anhydride.

Benzoylation of Caulosapogenin.—Tetrabenzoylcaulosapogenin was prepared by Power and Salway, but the present authors, in repeating the preparation of this compound, obtained, in addition, a second benzoyl derivative.

A quantity of caulosapogenin was benzoylated in pyridine solution, and the resulting product isolated. The latter was then dissolved in a mixture of alcohol and ether, and kept for some time, when it slowly crystallised. The solid so obtained, however, was found to be not homogeneous, and was therefore fractionally crystallised from ethyl acetate. A relatively small portion of the product was sparingly soluble, and separated from the hot solvent in stout, lustrous needles, melting constantly at 252°:

0·0914 gave 0·2628 CO_2 and 0·0686 H_2O. C = 78·4; H = 8·3 per cent.

This result does not agree with any possible benzoyl derivative of a substance, $C_{42}H_{66}O_6$. Nevertheless, the compound was undoubtedly pure, and regenerated pure caulosapogenin on hydrolysis. As in the case of the results obtained by the analysis of caulosapogenin methyl ether and its *benzoyl* derivative, described below, it indicates that further work is necessary before the formulæ of caulosapogenin and its derivatives can be considered to be definitely established. The amount of material available in the present case, however, was not sufficient to permit of this being accomplished.

The mother liquors from the above-described benzoyl derivative yielded tetrabenzoylcaulosapogenin, melting at 282°, and identical with the compound described by Power and Salway (*loc. cit.*). It separated in colourless prisms, and, on hydrolysis with alcoholic potassium hydroxide, regenerated pure caulosapogenin. (Found,

C = 77·4; H = 8·3. M.W. = 971. $C_{70}H_{82}O_{10}$ requires C = 77·6; H = 7·6 per cent. M.W. = 1082.)

In this case it will be noticed that the percentage of hydrogen found is appreciably higher than that required by theory.

Caulosapogenin Methyl Ether.—Caulosapogenin was dissolved in alcoholic sodium hydroxide and the solution heated under a reflux condenser, methyl iodide being added from time to time until a neutral liquid was obtained. A further quantity of alkali was then added, and the mixture again heated for some time, when the product was isolated. A substance was thus obtained which, after several crystallisations from methyl alcohol, formed long, soft needles, melting constantly at 229°:

0·3697,* on heating at 130°, lost 0·0107 CH_4O. CH_4O = 2·9.
0·1016 † gave 0·2849 CO_2 and 0·0961 H_2O. C = 76·5; H = 10·5.
0·0938 † „ 0·2627 CO_2 „ 0·0890 H_2O. C = 76·4; H = 10·5.
0·1692 „ 0·1037 AgI. OMe = 8·1.
0·0987 „ 0·0586 AgI. OMe = 7·8.

These results were amply confirmed, six concordant analyses being conducted; nevertheless, they are not in agreement with the results obtained by Power and Salway (*loc. cit.*), and do not harmonise with the theoretical figures for any possible methyl ether of caulosapogenin, providing that the latter is correctly represented by the formula $C_{42}H_{66}O_6$. A methyl ether of identical composition and properties was also obtained when caulosapogenin was methylated by means of methyl sulphate.

With the endeavour to elucidate the cause of the above discrepancies, a quantity of caulosapogenin, prepared by Power and Salway from *Caulophyllum thalictroides*, was methylated, and the resulting ether (m. p. 229°) analysed. The latter then gave C = 76·5; H = 10·5; OMe = 7·5 per cent., in fairly good agreement with the results given above, whilst Power and Salway found C = 75·7; H = 10·3; OMe = 5·5 per cent. It may be pointed out however, that the methoxyl found by the last-mentioned authors is nearly 1 per cent. higher than that theoretically required for caulosapogenin monomethyl ether.

The optical rotations of the methyl ethers of caulosapogenin from the two sources were identical, within the limits of experimental error. Thus, the present authors found for the product prepared from *Clematis* $[\alpha]_D + 73·3°$, in chloroform solution, whilst Power and Salway found $[\alpha]_D + 74·4°$.

Attempts were made to determine the molecular weight of caulosapogenin methyl ether in several solvents by the cryoscopic method, but resulted in failure, owing to the formation of colloidal

* Air dried. † Dried at 130°.

jellies. A determination was readily conducted, however, by the ebullioscopic method:

0·3353 in 23 of chloroform gave $\Delta t + 0·135°$. M.W. = 395.

This result, as well as that obtained from the benzoyl derivative of the methyl ether, described below, is much lower than was expected, with consideration of the results obtained from caulosapogenin and its benzoyl derivative.

Benzoyl Derivative of Caulosapogenin Methyl Ether.—Caulosapogenin methyl ether (m. p. 229°) was benzoylated in pyridine solution, and the resulting product dissolved in ether and deprived of pyridine and benzoic acid. The solution was then evaporated, and the residue kept for some time, when hard, colourless rosettes of needles slowly formed. The solid was then recrystallised from petroleum (b. p. 90—120°), when it formed needles, melting constantly at 187—188°:

0·0925 * gave 0·2640 CO_2 and 0·0716 H_2O. C = 77·8; H = 8·6.

0·4203 * in 23·96 benzene gave Δt −0·155°. M.W. = 566.

0·4695,* on hydrolysis, neutralised 13·9 c.c. $N/10$-NaOH. $CO{\cdot}C_6H_5 = 31·1$.

These results, as in the case of those obtained from the methyl ether, are not in harmony with any possible simple derivative of caulosapogenin, $C_{42}H_{66}O_6$.

The product resulting from the hydrolysis of the above-described *benzoyl* derivative consisted of the unchanged methyl ether, melting at 229°.

The amount of caulosapogenin available was not sufficient to conduct any further experiments with a view to arrive at an explanation of the above-described, apparently anomalous results.

A further portion of the aqueous solution of the solid (*a*) was boiled for two minutes with aqueous potassium hydroxide, when the mixture was cooled, acidified, and extracted with ether. The ethereal liquid was then fractionally extracted with various alkalis, when it yielded resinous products, together with small amounts of caulosapogenin and 3:4-dihydroxycinnamic acid.

The brown, resinous material which had separated from the aqueous solution of the solid (*a*), as previously mentioned, was carefully examined for the presence of caulosaponin, but with a negative result. Nevertheless, on hydrolysis with dilute sulphuric acid, it yielded a further quantity of caulosapogenin, together with dextrose, thus indicating the presence of a glucoside of caulosapogenin.

The filtrate (*b*) from the solid portion of the amyl alcohol

* Dried at 130°.

extract of the aqueous liquid was kept for some time in aqueous solution, but no solid substance separated. The liquid was therefore divided into two portions, which were heated with dilute aqueous sulphuric acid and potassium hydroxide respectively. The portion heated with acid yielded, on examination, a further amount of caulosapogenin, whilst that heated with the alkali gave about 3 grams of 3:4-dihydroxycinnamic acid. No other crystalline product could be isolated.

The original aqueous liquid (*A*) which had been extracted with amyl alcohol was deprived of this solvent, and treated with an excess of basic lead acetate. A voluminous, pale brown precipitate was thus produced, which was collected, washed, suspended in water, and decomposed by means of hydrogen sulphide. The filtrate from the lead sulphide precipitate could not be evaporated under diminished pressure on account of its saponaceous properties. It was therefore evaporated under the ordinary pressure, when a quantity of a brown solid separated, and was collected. This solid was found to consist of crude caulosapogenin, and readily yielded the latter in a pure state after solution in alcohol and treatment with animal charcoal.

The filtrate from the crude caulosapogenin above described, was dark brown. On treatment with alkali it yielded a small amount of 3:4-dihydroxycinnamic acid, together with amorphous products.

A further amount of caulosapogenin was obtained by extracting the above-mentioned lead sulphide precipitate with alcohol.

The filtrate from the basic lead acetate precipitate described above was bluish-green owing to the presence of copper. It was examined for the presence of asparagine and allantoin, but with a negative result. The lead and copper were then removed by means of hydrogen sulphide, and the filtered liquid concentrated under diminished pressure to the consistency of a syrup. On heating a portion of the syrup with potassium hydroxide it evolved a quantity of ammonia, and, when treated with phosphotungstic acid, it yielded a copious precipitate. This behaviour, however, was found to be due to the presence of ammonium salts, and not to betaine or choline.

A further portion of the aqueous syrup, when examined for sugar, readily yielded *d*-phenylglucosazone (m. p. 214°), and, after acetylation, gave β-penta-acetyldextrose, melting at 128—129°. It therefore evidently contained a considerable quantity of dextrose.

Examination of the Resin (B).

The resin (*B*) was a hard, dark green mass, and amounted to 420 grams, being thus equivalent to 2·29 per cent. of the weight of dried plant employed. It was dissolved in alcohol, mixed with purified sawdust, and the thoroughly dried mixture extracted successively, in a large Soxhlet apparatus, with light petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

Isolation of Melissic Acid and Myricyl Alcohol.

The petroleum extract of the resin was a dark green, waxy mass, and, when deprived of solvent, amounted to 300 grams. It was digested with 2 litres of ether, the mixture kept for some time, and filtered. Five grams of a green solid were thus obtained, which was washed with ether and then distilled under diminished pressure, when the greater part of it passed over at a high temperature as a light-coloured distillate. The latter, which solidified on cooling, was crystallised from ethyl acetate, but it did not appear homogeneous. It was therefore dissolved in chloroform, and the solution shaken with aqueous potassium hydroxide, when an insoluble potassium salt was precipitated. The latter was collected and washed by digestion with chloroform. The regenerated acid then separated from ethyl acetate in small, colourless crystals, which melted at 88°, and proved to be melissic acid. (Found, C=79·4; H=13·3. Calc., C=79·6; H=13·3 per cent.) Its identity was confirmed by the preparation of methyl melissate, which formed nacreous leaflets, melting at 74°.

The above-mentioned chloroform solution, after separation from the insoluble potassium salt, was washed and evaporated. The neutral residue was then crystallised from ethyl acetate, when it formed colourless leaflets, melting at 84°, and was identified as myricyl alcohol. (Found, C=82·0; H=14·2; Calc., C=82·2; H=14·2 per cent.

The ethereal solution of the more readily soluble constituents of the petroleum extract of the resin, from which the crude mixture of melissic acid and myricyl alcohol had been removed, was extracted with various alkalis, but nothing definite was obtained by this treatment. The ether was therefore removed, and the residue hydrolysed by heating for two hours with an excess of alcoholic potassium hydroxide. Water was then added, and the mixture extracted repeatedly with ether, during which operation

a quantity of a flocculent solid separated at the juncture of the aqueous and ethereal layers. This was collected, when it was found to consist chiefly of the potassium salt of a higher fatty acid. It was freed from some neutral material, and the fatty acid isolated and crystallised from ethyl acetate. Small, glistening leaflets were thus obtained, which melted at 79°, and were examined in connexion with another similar product, as described below.

Isolation of Ceryl Alcohol.

The ethereal solution of the unsaponifiable material, which had been separated from the alkaline aqueous liquid, as above described, was washed, dried, and evaporated. The residue (170 grams) was then dissolved in alcohol, when, on cooling, a quantity (about 45 grams) of an apparently amorphous product separated. This product was again dissolved in alcohol, and the material which separated on cooling collected. Since the solid so obtained did not appear homogeneous, it was dissolved in pyridine and the mixture heated with an excess of phthalic anhydride. Water was then gradually added to the warm solution, and the resulting liquid extracted with ether. The ethereal solution was deprived of pyridine and shaken with aqueous sodium carbonate, when a quantity of a solid sodium salt of an acid phthalic ester separated. The latter was collected and hydrolysed with alcoholic potassium hydroxide, when 5 grams of a fatty alcohol were obtained. The latter was recrystallised several times from petroleum (b. p. 90—120°) and ethyl acetate, when it formed small, glistening leaflets, melting constantly at 78°, and was identified as ceryl alcohol. (Found, C = 81·9; H = 14·1. Calc., C = 81·8; H = 14·1 per cent.)

Isolation of Hentriacontane, $C_{31}H_{64}$.

The ethereal solution from which the sodium salt of the ceryl hydrogen phthalate had been separated was evaporated and the residue (about 35 grams) distilled under diminished pressure. The distillate (about 15 grams) was crystallised from ethyl acetate, when it melted at 68°. The product, however, still contained some phytosterol and a little ceryl alcohol. The treatment with phthalic anhydride was therefore repeated, after which the neutral portion of the material was fractionally distilled under diminished pressure, and crystallised from ethyl acetate. Colourless, pearly leaflets were then obtained, which melted at 68°, and proved to be hentriacontane. (Found, C = 84·8; H = 14·7. Calc., C = 85·3; H = 14·7 per cent.)

Isolation of a Phytosterol.

The alcoholic solution of the unsaponifiable material, from which the crude mixture of ceryl alcohol and hentriacontane had been separated, was concentrated, and some ethyl acetate and a little water added. On keeping the mixture for some time 0·4 gram of colourless leaflets separated. This product had the properties of a phytosterol, but, as it did not appear to be homogeneous, it was converted into the acetyl derivative, and the latter crystallised several times alternately from alcohol and ethyl acetate. Colourless leaflets were then obtained, melting at 135°. The original substance was regenerated from the acetyl derivative, when 0·25 gram of colourless leaflets, melting at 154°, was obtained:

0·2353,* on heating at 130°, lost 0·0111 H_2O. $H_2O=4·7$.

0·0988 † gave 0·3046 CO_2 and 0·1060 H_2O. $C=83·4$; $H=11·9$.

$C_{27}H_{46}O$ requires $C=83·9$; $H=11·9$ per cent.

$C_{30}H_{50}O$ „ $C=84·5$; $H=11·7$ „

$C_{27}H_{46}O,H_2O$ requires $H_2O=4·5$ per cent.

$C_{30}H_{50}O,H_2O$ „ $H_2O=4·1$ „

The acetyl derivative prepared from the purified phytosterol melted as before, at 135°:

0·0760 gave 0·2274 CO_2 and 0·0773 H_2O. $C=81·6$; $H=11·3$.

$C_{29}H_{48}O_2$ requires $C=81·3$; $H=11·2$ per cent.

$C_{32}H_{52}O_2$ „ $C=82·1$; $H=11·1$ „

These results indicate the probability of the above-described phytosterol being a mixture of sitosterol and stigmasterol, but the amount available was too small to attempt the separation of the latter by means of the tetrabromoacetyl derivative.

Examination of the Fatty Acids.

The alkaline, aqueous liquid from which the unsaponifiable material had been removed, as above described, was acidified, and distilled in a current of steam, but only a trace of volatile acid was removed by this treatment. The mixture was then extracted with ether, when a quantity of a sparingly soluble solid separated, and was collected. The latter proved to consist of some myricyl alcohol, together with very crude phytosterolin, a further quantity of which was subsequently obtained, as described below. The dark green, ethereal solution containing the fatty acids was then evaporated to a small bulk and treated with light petroleum, when a quantity of chlorophyll was precipitated. The clear petroleum liquid was then evaporated, and the residual crude

* Air-dried. † Dried at 130°.

fatty acids converted into the methyl esters. After being purified by distillation under diminished pressure, the latter, which amounted to 122 grams, were hydrolysed, and the recovered acids separated into their saturated and unsaturated components by means of the lead salts, in the usual manner.

The Saturated Fatty Acids.—The saturated fatty acids, which amounted to 43 grams, were converted into their methyl esters and the latter fractionally distilled several times under diminished pressure, when the following fractions were collected: (i) below 200° (4 grams); (ii) 200—205° (7·6 grams); (iii) 205—210° (6·7 grams); (iv) 210—215° (3·4 grams); (v) 215—225° (2·7 grams); (vi) 225—235° (1·8 grams); (vii) 235—270° (3·2 grams); (viii) above 270° (3·0 grams)/20 mm.

Fractions (i) and (ii) solidified on cooling, and were found to consist of methyl palmitate. They yielded palmitic acid, melting at 63°. (Found, C=74·8; H=12·6. Calc., C=75·0; H=12·5 per cent.) Fraction (iii) consisted of slightly impure methyl palmitate, whilst fraction (iv) yielded a mixture of palmitic and stearic acids, melting at 54°. Fractions (v) and (vi) gave acids melting at 64·5° and 66° respectively, but they were probably not homogeneous.. Fraction (vii), on hydrolysis, yielded an acid which, after being crystallised three times from ethyl acetate, appeared quite homogeneous. It formed glistening plates melting at 69·5°. It was reconverted into the methyl ester, and the latter crystallised, when nacreous leaflets, melting sharply at 51°, were obtained:

0·0999 gave 0·2849 CO_2 and 0·1180 H_2O. C=77·8; H=13·1.

After being again crystallised from ethyl acetate the melting point was unchanged:

0·1013 gave 0·2889 CO_2 and 0·1198 H_2O. C=77·8; H=13·1.

$C_{23}H_{46}O_2$ requires C=78·0; H=13·0 per cent.

This ester was then again hydrolysed, and the resulting acid crystallised three times from ethyl acetate, when the melting point remained unchanged, at 69·5°:

0·0899 gave 0·2551 CO_2 and 0·1055 H_2O. C=77·3; H=13·1.

$C_{22}H_{44}O_2$ requires C=77·4; H=13·0 per cent.

The above-described acid is thus seen to agree in composition with behenic acid, which is stated to melt at 82—84°. It would therefore appear to be an isomeride of the latter, and if this be the case, it is a new compound.

Isolation of Cerotic Acid.

Fraction (viii) of the esters, which distilled above 270°, was hydrolysed by means of alcoholic potassium hydroxide, and the product freed from traces of unsaponifiable matter. The acid was then isolated, and crystallised twice from acetic acid, when it formed small, colourless leaflets, melting at 79°. It was found to be identical with the acid melting at 79° which was obtained from the previously-described, sparingly soluble potassium salt, and the two products were therefore mixed. In order to ensure its purity, the total acid was again converted into the methyl ester, and the latter crystallised, after which it melted at 63°. The acid was then regenerated, when it melted at 79°, and proved to be cerotic acid. (Found, C = 78·7; H = 13·2. Calc., C = 79·0; H = 13·2 per cent.)

The Unsaturated Fatty Acids.—The unsaturated acids were regenerated from the lead salts soluble in ether and converted into their methyl esters, which amounted to 63 grams. The latter were fractionally distilled under 20 mm. pressure, when the following fractions were obtained: (i) Below 215° (14·5 grams, iodine value 143·3); (ii) 215—218° (10·5 grams, iodine value 180·0); (iii) 218—222° (23·7 grams, iodine value 189·8); (iv) 222—226° (7·7 grams, iodine value 187·2).

Fraction (iii) gave C = 77·6; H = 11·4.

$C_{19}H_{34}O_2$ requires C = 77·6; H = 11·6 per cent. I.V. = 172·7.

It thus appears that the unsaturated acids consisted chiefly of linolic acid, together with a small amount of an acid of a higher degree of unsaturation.

Ether Extract of the Resin.

Isolation of a Phytosterolin, $C_{36}H_{60}O_6$.

The ether extract of the resin was dark green, and amounted to 49 grams. A portion of it (15 grams) which was very sparingly soluble in ether was dissolved in boiling alcohol, and the solution concentrated, when about 0·5 gram of solid separated, and was removed from the hot liquid. This solid, after purification by means of animal charcoal, melted at 295°, and possessed the properties of a phytosterolin (phytosterol glucoside). It was heated with acetic anhydride, when an acetyl derivative was obtained which formed colourless leaflets, melting at 149°:

0·1011 gave 0·2574 CO_2 and 0·0834 H_2O. C = 69·4; H = 9·2.

$C_{36}H_{56}O_6(CO{\cdot}CH_3)_4$ requires C = 69·8; H = 9·0 per cent.

It therefore appears that this phytosterolin consists essentially

of the glucoside of stigmasterol, $C_{30}H_{50}O$, or an isomeric alcohol which, as already mentioned, appears to occur in *Clematis* in the free state.

Isolation of a Glucoside of Caulosapogenin.

The portion of the ether extract of the resin which was readily soluble in ether was extracted with aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide. During these operations considerable quantities of a sparingly soluble, green powder were thrown out of solution, but the only definite compound that could be isolated from this material was a further small amount of myricyl alcohol. The material dissolved by the ammonium carbonate and potassium hydroxide was amorphous, and small in amount, but on acidifying the sodium carbonate extract and shaking it with ether, a quantity of a sparingly soluble solid remained undissolved. The latter was collected and crystallised from alcohol with the employment of animal charcoal, when it formed colourless, microscopic crystals, which melted and decomposed at 235—240°:

0·1420,* on heating at 130°, lost 0·0129 H_2O. $H_2O = 9·1$.
0·1016 * gave 0·2222 CO_2 and 0·0830 H_2O. $C = 59·7$; $H = 9·1$.
0·0892 † „ 0·2128 CO_2 „ 0·0720 H_2O. $C = 65·1$; $H = 9·0$.
$C_{54}H_{86}O_{16},5\frac{1}{2}H_2O$ requires $C = 59·5$; $H = 8·9$; $H_2O = 9·1$ per cent.
$C_{54}H_{86}O_{16}$ requires $C = 65·5$; $H = 8·7$ per cent.

This *substance* had the properties of a saponin, since its solution, on agitation, yielded a strong, persistent froth. On hydrolysis with dilute sulphuric acid it yielded caulosapogenin, $C_{42}H_{66}O_6$, and dextrose, according to the following equation:

$$C_{54}H_{86}O_{16} + 2H_2O = C_{42}H_{66}O_6 + 2C_6H_{12}O_6.$$

It is, however, not identical with caulosaponin (Power and Salway, *loc. cit.*), which melts at 250—255°, and with which it was directly compared. The latter glucoside, although it also yields caulosapogenin and two molecules of dextrose on hydrolysis, possesses the formula $C_{54}H_{88}O_{17}$ when dried at 130°, and therefore appears persistently to retain 1 molecule of water of crystallisation.

The neutral portion of the ether extract of the resin, which remained dissolved in the ether after extraction with alkalis, was small in amount, and nothing definite could be obtained from it.

* Dried in the air. † Dried at 130°.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

The chloroform, ethyl acetate, and alcohol extracts of the resin amounted to 9, 10, and 37 grams respectively. They all consisted of dark-coloured, amorphous resins, and nothing definite could be isolated from any of them.

Summary.

The results of the foregoing investigation may be summarised as follows:

The material employed consisted of the flowering branches of *Clematis vitalba*, Linné, which had been specially collected for the purpose.

Preliminary tests showed the absence of any alkaloid, and that only a trace of volatile material was present.

An alcoholic extract of the dried and ground material yielded, in addition to much chlorophyll and resin, the following definite compounds: (i) 3:4-Dihydroxycinnamic acid; (ii) caulosapogenin, $C_{42}H_{66}O_6$, identical with the substance recently isolated by Power and Salway from *Caulophyllum thalictroides*. Some of the derivatives of caulosapogenin yielded, on analysis, apparently anomalous results, which cannot at present be explained; (iii) a *saponin*, $C_{54}H_{86}O_{16}$, which proved to be a glucoside of caulosapogenin; (iv) dextrose; (v) myricyl and ceryl alcohols; (vi) hentriacontane, $C_{31}H_{64}$; (vii) a phytosterol, which appeared to consist of a mixture of sitosterol, $C_{27}H_{46}O$, and stigmasterol, $C_{30}H_{50}O$; (viii) a phytosterolin, which apparently consisted essentially of stigmasterol glucoside; (ix) melissic, cerotic, and palmitic acids, together with a mixture of unsaturated acids consisting largely of linolic acid, and an acid, $C_{22}H_{44}O_2$ (m. p. 69·5°), apparently isomeric with behenic acid.

The statements regarding the irritant properties of *Clematis vitalba* cannot be confirmed.

The Wellcome Chemical Research Laboratories,
London, E.C.

R. Clay and Sons, Ltd., Brunswick St., Stamford St., S.E., and Bungay, Suffolk.